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A common co-morbidity modulates disease expression and treatment efficacy in inherited cardiac sodium channelopathy

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Abstract

Aims: Management of patients with inherited cardiac ion channelopathy is hindered by variability in disease severity and sudden cardiac death (SCD) risk. We here investigated the modulatory role of hypertrophy on arrhythmia and SCD risk in sodium channelopathy.

Methods and Results: Follow-up data was collected from 164 individuals positive for the *SCN5A*-1795insD founder mutation and 247 mutation-negative relatives. A total of 38 (obligate) mutation-positive patients died suddenly or suffered life-threatening ventricular arrhythmia. Of these, 18 were aged >40 years, a high proportion of which had a clinical diagnosis of hypertension and/or cardiac hypertrophy. While pacemaker implantation was highly protective in preventing bradycardia-related SCD in young mutation-positive patients, 7 of them aged >40 experienced life-threatening arrhythmic events despite pacemaker treatment. Of these, 6 had a diagnosis of hypertension/hypertrophy, pointing to a modulatory role of this co-morbidity. Induction of hypertrophy in adult mice carrying the homologous mutation ($Scn5a^{1798insD/+}$) caused SCD and excessive conduction disturbances, confirming a modulatory effect of hypertrophy in the setting of the mutation. The deleterious effects of the interaction between hypertrophy and the mutation were prevented by genetically impairing the pro-hypertrophic response and by pharmacological inhibition of the enhanced late sodium current associated with the mutation.

Conclusion: This study provides the first evidence for a modulatory effect of co-existing cardiac hypertrophy on arrhythmia risk and treatment efficacy in inherited sodium channelopathy. Our findings emphasize the need for continued assessment and rigorous treatment of this co-morbidity in *SCN5A* mutation-positive individuals.

Key words

SCN5A

Sudden death

Cardiac hypertrophy

Hypertension

Conduction delay

Ventricular arrhythmias

Translational perspectives

SCN5A mutations present with a broad spectrum of clinical phenotypes, including sudden cardiac death (SCD). Disease penetrance and severity varies between individuals carrying the same *SCN5A* mutation, and common co-morbidities may modulate arrhythmia and SCD risk with potential implications for anti-arrhythmic treatment efficacy. This study conducted in patients and mice, provides strong evidence for a modulatory role of hypertension/hypertrophy in modulating arrhythmic risk of the *SCN5A*-1795insD mutation. Our findings support the concept that *SCN5A* mutation-positive patients should be carefully monitored for the development of cardiac hypertension and hypertrophy during follow up, and that their clinical management should be adjusted where necessary to also include rigorous management of this co-morbidity.

Abbreviations list

Scn5a/SCN5A	gene encoding the alpha subunit of the cardiac sodium channel
WT	wild type for the *Scn5a* gene
MUT	mutant for the *Scn5a* gene (*Scn5a*$^{1798insD/+}$)
TAC	transverse aortic constriction
ECG	electrocardiogram
PM	pacemaker
ICD	implantable cardioverter defibrillator
SCD	sudden cardiac death
$I_{Na,L}$	late component of the sodium current
RAN	Ranolazine

Introduction

The genetic basis of the Mendelian cardiac rhythm disorders associated with sudden cardiac death (SCD) has been brought into focus over the last 20 years with the discovery of a large spectrum of causal mutations in genes encoding components of cardiac ion channels.[1] Although this enabled genetic testing and consequently improvements in clinical care, patient management is still hindered by the reduced penetrance and substantial variability in disease severity and SCD risk among mutation-positive individuals.[1] Although co-morbidities are expected to modulate disease severity, these remain unexplored. Identification of disease modifiers in these disorders is however hindered by the substantial genetic heterogeneity across patients, as different mutations may be associated with different effects and thus also contribute to inter-individual variability.[2] Studies in large families that harbour founder mutations (where all affected individuals carry the same familial mutation) circumvent this limitation and therefore offer opportunities for the identification of modulatory factors.

We have previously described a large Dutch family harboring a founder mutation, p.Tyr1795_Glu1796insAsp (previously called '*SCN5A*-1795insD'), in the *SCN5A* gene which encodes the major sodium channel isoform in heart ($Na_V1.5$).[3,4] This mutation displays multiple biophysical defects and causes 'overlap' sodium channelopathy with features of long QT syndrome, Brugada syndrome, and conduction disease.[5,6] While variability in disease severity among mutation-positive individuals in this family is long recognized,[3] predictors of arrhythmia and SCD risk have remained elusive. By combining clinical observations in the family with experimental studies in *Scn5a*$^{1798insD/+}$ mice carrying the mouse homolog of the mutation, we here uncovered a modulatory effect of hypertension and cardiac hypertrophy on disease severity

and expression. In particular, the co-occurrence of cardiac hypertrophy was found to exacerbate cardiac conduction slowing and arrhythmia risk in the setting of the mutation, and was associated with a decreased efficacy of pacemaker treatment in preventing SCD. Our findings furthermore provide evidence for a potential therapeutic role of pharmacological late sodium current inhibition.

Methods

Details on study approval, *SCN5A*-1795insD mutation study population, clinical data collection, pathology reports, generation of *Scn5a*$^{1798insD/+}$ and *Scn5a*$^{1798insD/+}$-*Nfatc2*$^{-/-}$ mice, transverse aortic constriction (TAC) and chow experiments, electrophysiological assessments in Langendorff-perfused hearts, quantitative PCR assay and analysis and whole-mount *in situ* hybridization, and statistical analysis are provided in the Supplementary Methods. Data are presented as mean±SD or median(interquartile range), as appropriate.

Results

Hypertension and hypertrophy in older SCN5A-1795insD mutation-positive patients who died suddenly

Through an extensive genealogical search, we reconstructed the pedigree of the family with the *SCN5A*-1795insD mutation back to the 18th century, linking 164 mutation-positive individuals and 247 mutation-negative relatives (Figure 1A). Mutation-positive patients displayed (atrio-)ventricular conduction slowing, sinus node dysfunction, excessive ventricular repolarization abnormalities at slow heart rates, and SCD occurring predominantly at night.[3,4] A total of 38 individuals died suddenly or suffered life-threatening ventricular arrhythmia (13 males, 25 females; average age at event 38±18 years, range 13-76) (Figure 1A,B). Twenty-five of these individuals were confirmed or obligate mutation-positive; genetic testing was not possible in the rest as these were deceased individuals from past generations, the majority of which died more than 50 years ago. Eighteen individuals who suffered SCD or a life-threatening arrhythmia were older than 40 years at the time of the event (4 males, 14 females, average age 54±9 years, range 41-76) (Figure 1A,B). Recent clinical information was available for 10 of these (all confirmed mutation-positive); 9 of them had a clinical diagnosis of hypertension and/or evidence for the presence of left ventricular hypertrophy (including increased heart weight) on MRI, echocardiography or post-mortem examination (Table 1), suggesting a modulatory effect of cardiac hypertrophy on arrhythmia risk. Post-mortem analysis furthermore indicated the additional presence of slight interstitial fibrosis (potentially hypertension-related) in 3 out of 4 patients (Table 1). Evidence for coronary artery disease, with hypertension as a major risk factor, was found in 2 patients. In one patient, small foci of subendocardial necrosis were found, but this

was not associated with critically stenosing or thrombosed coronary lesions. The other patient displayed a fibrotic scar consistent with an old myocardial infarction without signs of recent-onset ischemia.

Decreased pacemaker treatment efficacy in SCN5A-1795insD mutation-positive patients older than 40

Mutation-positive patients died suddenly predominantly during the night, and excessive QT-prolongation during (nocturnal) bradycardic episodes was frequently documented on Holter recordings.[3,4,7] Hence, a bradycardia-dependent trigger for arrhythmia was originally suspected, and pacemaker implantation has been routinely employed in the family to prevent SCD.[4] While this approach initially proved successful,[4] in the last decade 7 mutation-positive individuals suffered ventricular tachyarrhythmias and/or SCD despite pacemaker implantation (Figure 1A). All of them were older than 40 (age range 41-58 years), and in the majority, ventricular fibrillation or tachycardia was documented (Table 1), in addition to ventricular extrasystoles recorded during Holter monitoring or exercise testing in several cases. Figure 1D shows a pacemaker readout showing a nocturnal, fast ventricular tachycardia (presumably polymorphic) in a 58-year old female mutation-positive patient; following this episode, her pacemaker was replaced with an ICD. These observations suggested an age-dependent shift towards a different arrhythmia triggering mechanism at older age with arrhythmias occurring despite prevention of bradycardia. Indeed, comparing the occurrence of SCD/life-threatening arrhythmia between individuals with and without pacemaker showed that while pacemaker implantation was highly protective in young mutation-positive patients, its efficacy in preventing arrhythmias and SCD was significantly decreased above the age of 40 years (p=0.028; proportional hazard assumption

of pacemaker implantation tested using the Schoenfeld residuals from a Cox regression model) (Figure 1C). Importantly, in 6 of the 7 mutation-positive patients who suffered a life-threatening event despite pacemaker treatment, a history of hypertension was confirmed and/or the presence of left ventricular hypertrophy documented on MRI, echocardiography, or post-mortem examination (Table 1). These observations led us to hypothesize that the co-occurrence of the mutation with cardiac hypertrophy, developing with age secondary to for instance hypertension, plays a pivotal role in modulating arrhythmia risk.

The co-occurrence of cardiac hypertrophy is pro-arrhythmic in aged Scn5a$^{1798insD/+}$ mice

We further explored the modulatory role of cardiac hypertrophy in *Scn5a*$^{1798insD/+}$ mice carrying the exact mouse homolog of the human *SCN5A*-1795insD mutation.[5,8] We have previously generated two distinct mouse lines harbouring the *Scn5a*$^{1798insD/+}$ mutation, with respectively the FVB/N and 129P2 inbred genetic background, enabling investigation of the effect of the mutation on different genetic backgrounds. In young adult mice we previously demonstrated strain-dependent variable disease severity, with more pronounced conduction slowing and prolongation of repolarization in mutant mice of the 129P2 strain.[8] We now studied aged wild-type (WT) and *Scn5a*$^{1798insD/+}$ mutant (MUT) mice of both strains. The 129P2 strain was found to develop more cardiac hypertrophy with age as compared to the FVB/N strain. This feature was intrinsic to the 129P2 strain and independent of the mutation, since both aged WT- and MUT-129P2 mice displayed greater heart weight and higher expression levels of pro-hypertrophic markers as compared to aged WT- and MUT-FVB/N mice (Figure 2A,B). These intrinsic strain-dependent differences in susceptibility to hypertrophy enabled a comparison of the pro-arrhythmic effect of the mutation in the presence (i.e. aged 129P2-MUT mice) and absence (i.e.

aged FVB/N-MUT mice) of hypertrophy. We therefore conducted ECG studies in anesthetized mice and electrophysiological studies in Langendorff-perfused hearts, comparing aged (8-22 months old) WT and MUT mice of both strains. This indeed uncovered a genotype-strain interaction, where aged 129P2-MUT mice displayed significantly more pronounced ventricular conduction slowing *in vivo* (QRS-duration on ECG analysis; Figure 2C,D; Supplementary Table 1) and *ex vivo* (LV activation time in isolated Langendorff-perfused hearts; Figure 2F,G). Moreover, 129P2-MUT mice exhibited significantly more spontaneous ventricular extrasystoles and arrhythmias *in vivo* (Figure 2C,E) and an increased inducibility of ventricular arrhythmias *ex vivo* (Figure 2H,I). While these observations do not provide a causal link, they are in line with a pro-arrhythmic interaction between hypertrophy and the mutation, similar to our observations in the *SCN5A*-1795insD family.

Chronic pressure overload elicits conduction delay and sudden death in Scn5a$^{1798insD/+}$ mice

To provide direct evidence for a modulatory effect of hypertrophy in the setting of the mutation, we subjected adult FVB/N-WT and FVB/N-MUT mice (10-12 weeks old) to transverse aortic constriction (TAC; duration of 2 weeks), an intervention which leads to chronic pressure overload and consequent development of cardiac hypertrophy. TAC induced similar extent of hypertrophy in WT and MUT mice, as illustrated by equal increases in heart mass and upregulation of hypertrophic genes (Figure 3B,C; Supplementary Table 2). However, approximately 35% of MUT-TAC mice died suddenly during the 2-week post-TAC period, while all WT-TAC and sham mice survived (Figure 3A). Continuous 24-hour telemetric ECG recordings in a subset of MUT-TAC mice revealed progressive bradycardia and excessive (atrio-)ventricular conduction abnormalities prior to SCD (Supplementary Figure 2). ECG analysis in

surviving MUT mice post-TAC uncovered a more pronounced increase in QRS-duration compared to WT (Supplementary Table 3). Moreover, *ex vivo* measurements in isolated Langendorff-perfused hearts post-TAC showed atrio-ventricular delay and exacerbated ventricular conduction slowing in MUT but not WT mice (Figure 3D-G; Supplementary Table 2). Hence, TAC elicited SCD and conduction abnormalities in MUT mice only, indicating a synergistic, deleterious interaction between cardiac hypertrophy and the mutation.

TAC-induced conduction abnormalities and SCD in Scn5a$^{1798insD/+}$ mice are attenuated by decreasing the hypertrophic response through genetic inhibition of the calcineurin-Nfat pathway

Activation of the calcineurin-Nfat (Nuclear Factor of Activated T-cells) signaling pathway is known to play a major role in mediating the pro-hypertrophic consequences of chronic pressure overload of the heart. To investigate whether the more severe electrophysiological abnormalities in FVB/N-MUT mice post-TAC are the direct consequences of cardiac hypertrophy and not to other (indirect) effects of pressure overload, we abrogated the hypertrophic response by inducing genetic deletion of the main downstream effector of the calcineurin-Nfat pathway by crossing *Scn5a$^{1798insD/+}$* mice with mice lacking *Nfatc2* (*Nfatc2$^{-/-}$*).[9,10] WT and MUT (*Scn5a$^{1798insD/+}$*) mice deficient for *Nfatc2* (*Nfatc2$^{-/-}$*), and littermate WT and MUT animals with unaltered *Nfatc2* expression (*Nfatc2$^{+/+}$*), were subjected to TAC for a period of 2 weeks. As expected, cardiac hypertrophy in response to pressure overload was attenuated in WT and MUT mice deficient for *Nfatc2* (WT-*Nfatc2$^{-/-}$* and MUT-*Nfatc2$^{-/-}$*), as illustrated by lower heart weights and lower expression of hypertrophic genes as compared to WT-*Nfatc2$^{+/+}$* and MUT-*Nfatc2$^{+/+}$* (Figure 4B,C, Supplementary Table 4). No SCD was observed in MUT-*Nfatc2$^{-/-}$* mice subjected to TAC (Figure 4A), and the (atrio-)ventricular conduction abnormalities secondary to TAC observed in

MUT mice with intact *Nfatc2* expression were rescued in MUT-*Nfatc2*$^{-/-}$ mice (Figure 4D-F; Supplementary Table 4). Hence, blocking the downstream, pro-hypertrophic signaling cascade prevented the TAC-induced conduction abnormalities and SCD in MUT mice, providing support for a direct interaction between the mutation and cardiac hypertrophy.

Chronic late sodium current inhibition prevents TAC-induced conduction abnormalities and SCD in Scn5a$^{1798insD/+}$ *mice*

We have previously demonstrated that the *SCN5A*-1795insD mutation is associated with multiple biophysical defects including a gain of channel function due to sustained (late) inward sodium current.[5,6] This mutation-induced enhanced late sodium current ($I_{Na,late}$) can be blocked through pharmacological inhibition. We therefore explored whether blocking this biophysical defect of the mutation would prevent the exacerbation of electrophysiological abnormalities associated with TAC in MUT mice. For this we administered the $I_{Na,late}$ inhibitor Ranolazine by feeding WT and MUT mice either control or Ranolazine chow for the 2-week period of TAC or sham (starting 2 days after the TAC or sham procedure). Food intake and body weights were constant throughout the duration of the experiment and did not differ between groups (Supplementary Figure 1). $I_{Na,late}$ inhibition decreased QTc-duration in sham mice, and moreover prevented TAC-induced QTc-prolongation (Supplementary Table 3). Blocking $I_{Na,late}$ did not affect the magnitude of TAC-induced cardiac hypertrophy, as indicated by a similar increase in pro-hypertrophic markers in WT-TAC and MUT-TAC mice that were fed Ranolazine chow (Figure 4B,C; Supplementary Table 2). Yet, $I_{Na,late}$ blockade prevented SCD and attenuated (atrio-Supplementary Table 2). Hence, pharmacological inhibition of the mutation-induced enhanced)ventricular conduction abnormalities in MUT mice subjected to TAC (Figure 4D-F, $I_{Na,late}$

rescued the TAC-induced deleterious interaction between hypertrophy and the *Scn5a*$^{1798insD/+}$ mutation.

Discussion

Our findings point to a modulatory effect of hypertension and consequent cardiac hypertrophy on age-dependent risk for sudden arrhythmic death and pacemaker treatment efficacy in the *SCN5A*-1795insD mutation-positive patients. This is supported by our observations in *Scn5a*$^{1798insD/+}$ mice carrying the homologous mutation, where cardiac hypertrophy (either occurring with age or induced by TAC) led to severe conduction disturbances and an increased risk for ventricular arrhythmias and/or SCD. This study for the first time provides evidence for a modulatory role of co-morbidity in modulating disease severity of an inherited arrhythmic disease, demonstrating a pro-arrhythmic gene-environment interaction.

Modulatory effect of cardiac hypertrophy on (age-dependent) arrhythmic phenotype

Cardiac hypertrophy, which commonly occurs as a consequence of hypertension, develops over time and progressively remodels the myocardium. Its impact is therefore expected to increase with age, potentially altering disease severity in mutation-positive patients in an age-dependent manner. The observed age-dependent shift from bradycardia-induced (prevented by pacemaker therapy) to apparent bradycardia-independent arrhythmias and SCD in older *SCN5A*-1795insD mutation-positive patients moreover indicates a modulatory effect on disease expression. Our clinical findings are further supported by our observations in *Scn5a*$^{1798insD/+}$ mice, where mice from the strain most prone to age-dependent hypertrophy (i.e. 129P2-*Scn5a*$^{1798insD/+}$ mice) developed a more pronounced arrhythmic phenotype with significantly more spontaneous and inducible ventricular arrhythmias. Although other factors besides hypertrophy, that differ between the patients and between the two mouse strains may also contribute, collectively this

human and mouse data support the concept that age-dependent development of cardiac hypertrophy interacts with sodium channel dysfunction to predispose the heart to ventricular arrhythmias and SCD. Data obtained in the TAC studies in young-adult *Scn5a*$^{1798insD/+}$ mice provide direct proof of the modulatory role of hypertrophy on disease expression. Despite the fact that wild type and *Scn5a*$^{1798insD/+}$ mice developed similar extent of cardiac hypertrophy secondary to TAC, SCD and conduction abnormalities were observed only in *Scn5a*$^{1798insD/+}$ mice with chronic pressure overload. In contrast to aged 129P2-*Scn5a*$^{1798insD/+}$ mice however, no spontaneous or inducible ventricular arrhythmias were observed in mutant mice following TAC (data not shown). The young-adult age of the mice subjected to TAC, the relatively abrupt development of hypertrophy secondary to TAC (in contrast to gradual progression with age), and the short duration of TAC (two weeks) may underlie this apparent discrepancy. One should note however that as for aged *Scn5a*$^{1798insD/+}$ mice, young-adult *Scn5a*$^{1798insD/+}$ mice developed ventricular conduction slowing post-TAC, a phenomenon that is well established to promote ventricular arrhythmias.

Potential mechanisms underlying modulatory role of hypertrophy

The mechanisms underlying the modulatory, pro-arrhythmic effects of hypertension and consequent hypertrophy may be numerous and complex. Hypertrophy is associated with progressive electrical (alterations in sodium current and other ion channels), homeostatic (dysregulation of intracellular calcium homeostasis) and structural (collagen deposition) remodelling.[11–14] These alterations may be further exacerbated in the setting of an *SCN5A* mutation, acting synergistically with the biophysical defects caused by the *SCN5A* mutation in creating a highly arrhythmogenic environment.[15,16] In support of this, we found that similar

levels of hypertrophy induced a pro-arrhythmic phenotype in *Scn5a*$^{1798insD/+}$ but not wild type mice, indicating a synergistic interaction between hypertrophy and the mutation. Moreover, TAC-induced conduction disturbances and SCD were prevented by either blocking the downstream pro-hypertrophic response (by genetic deletion of *Nfatc2*) or by pharmacological inhibition of the detrimental consequences of the mutation (i.e. through $I_{Na,late}$ inhibition). Enhanced $I_{Na,late}$ increases sodium influx, which may secondarily lead to increased intracellular calcium concentrations. The latter is a well-established pro-arrhythmic feature of hypertrophy and heart failure, and may be exacerbated in *SCN5A*-1795insD mutation-positive patients in the presence of hypertrophy.[17,18] The involvement of the enhanced $I_{Na,late}$ defect in mediating, at least in part, the observed interaction with hypertrophy is supported by the fact that targeting this molecular defect by $I_{Na,late}$ blockade prevented SCD and attenuated (atrio-)ventricular conduction abnormalities in mutant mice post-TAC. While enhanced $I_{Na,late}$ is a well-established pro-arrhythmic consequence of hypertrophy,[18] one might speculate that other hypertension-associated pathways (including the renin angiotensin aldosterone system) may also be involved in mediating the role of hypertrophy in increasing arrhythmic propensity, with potential relevance for patient management.

Implications for age-dependent treatment efficacy

The observed age-dependent change in arrhythmia phenotype had crucial consequences for treatment efficacy in *SCN5A*-1795insD mutation-positive patients: while pacemaker implantation remained 100% efficacious in preventing SCD in young mutation-positive individuals, it no longer afforded complete protection over the age of 40. These findings therefore underline the necessity for additional treatment strategies in older *SCN5A* mutation-positive patients. Our

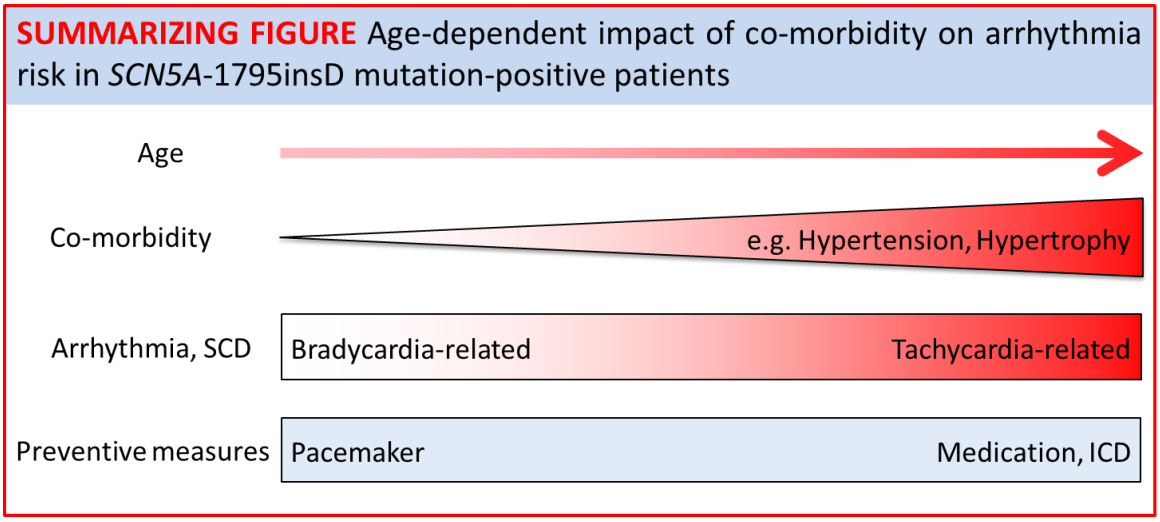
combined observations in the family and in mice implicate a role for hypertension and consequent hypertrophy in disease expressivity with consequences for treatment efficacy, although other co-morbidities may also play an as yet unidentified modulatory role. Our findings indicate a pro-arrhythmic interaction between hypertrophy and the mutation, and prevention of either hypertrophy (by genetic deletion of Nfatc2) or the effect of the mutation (by Ranolazine) was sufficient to prevent SCD in mutant mice subjected to TAC. Hence, clinical management of affected patients with either $I_{Na,late}$ inhibition or anti-hypertensive treatment (aimed at preventing LVH) is potentially beneficial, since either of these approaches would prevent the deleterious interaction between cardiac hypertrophy and the mutation. While $I_{Na,late}$ inhibition may have additional (long-term) benefits aside from restoration of repolarization, including the prevention of intracellular sodium/calcium dysregulation, it is as yet unclear whether this therapeutic approach will be clinically applicable in the near future. Ranolazine may have pro-arrhythmic side-effects due to its (limited) I_{Kr} blocking properties, and the development of more selective $I_{Na,late}$ inhibitors (including Eleclazine) was discontinued.[19,20] Thus, while awaiting (further) development of novel compounds targeting $I_{Na,late}$,[20] current clinical management should focus on carefully monitoring *SCN5A*-1795insD mutation-positive patients for co-morbidities such as hypertension. In addition, hypertension should be aggressively treated early on to prevent LVH development, which should be regularly monitored by echocardiography. In particular, drugs targeting the renin-angiotensin system may be beneficial.[21] This approach is in line with the 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death,[REF] which state that appropriate therapy should also take into account underlying diseases, co-morbidities, and associated medical conditions that may contribute to and/or exacerbate arrhythmia.

Limitations

While the homogeneity in genetic cause in the extended *SCN5A*-1795insD family allowed for detection of this interaction, it is associated with the limitation that transferability of these observations to other *SCN5A* mutations, although considered likely, will need to be explored. It is possible that our current findings are specific for "overlap syndrome" mutations associated with both a loss and a gain of sodium channel function. Furthermore, while hypertension and/or hypertrophy was observed in the majority of older *SCN5A*-1795insD mutation-positive patients who suffered a life-threatening event despite pacemaker treatment, we currently do not know the exact prevalence of these co-morbidities in all mutation-positive and -negative individuals in the family.

Conclusion

Our findings show that a common co-morbidity (e.g. hypertension) may significantly affect arrhythmia risk and survival in inherited sodium channelopathy. The impact of such unrelated co-morbidities varies with age, leading to potential age-dependent changes in arrhythmia mechanism. As a consequence, efficacy of treatment strategies to prevent SCD in the setting of inherited arrhythmic disease may vary over time, underscoring the need for continuous diagnosis and monitoring of relevant co-morbidities and their rigorous treatment.



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Conflict of interest:

S.R. and L.B. are former employees of Gilead Sciences. A.A.M.W. serves on the scientific advisory board of Lilanova. Other authors report no conflicts.

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Figure legends

Figure 1. Life-threatening cardiac events in *SCN5A*-1795insD mutation-positive patients. (**A**) The *SCN5A*-1795insD founder population pedigree. Circles indicate *SCN5A*-1795insD mutation-positive patients suffering sudden cardiac death (SCD), ventricular fibrillation (VF), and/or ventricular tachycardia (VT) in the presence or absence of pacemaker (PM) treatment and hypertension and/or left ventricular hypertrophy (HYP). (**B**) Survival from SCD and/or ventricular arrhythmias was significantly reduced in *SCN5A*-1795insD mutation-positive patients (n=164) versus mutation-negative relatives (n=247) (p=1.3026E-18). (**C**) Overall survival from SCD and/or ventricular arrhythmias in *SCN5A*-1795insD mutation-positive patients was significantly higher in those with (n=87) as compared to without (n=77) pacemaker (p=0.000003), but pacemaker efficacy was significantly reduced in mutation-positive patients above the age of 40 years (p=0.028; Cox regression model). (**D**) Pacemaker readout (A:atrial lead; V:ventricular lead; paper speed 25 mm/sec) of a 58-year female mutation-positive patient showing a nocturnal, fast (up to 300/min) and apparently polymorphic VT despite adequate anti-bradycardia treatment.

Figure 2. Pro-arrhythmic effects of cardiac hypertrophy in aged *Scn5a*$^{1798insD/+}$ mice. Mice of the 129P2 strain develop more severe cardiac hypertrophy with age (WT, wild type and MUT, *Scn5a*$^{1798insD/+}$ to a similar extent) than mice of the FVB/N strain, as indicated by increased heart weight to body weight ratio (**A**) and *Anf* expression on *in situ* hybridization (**B**). (**C**) Typical examples of surface ECGs and arrhythmias. (**D,E**) Aged 129P2-MUT mice show significantly increased QRS-duration and more spontaneous arrhythmias (Pearson Chi-square overall

p=0.000039) on ECG than aged 129P2-WT, FVB/N-WT and FVB/N-MUT mice (SND: sinus node dysfunction; AVB: atrio-ventricular block; VPBs: ventricular premature beats; VT: ventricular tachycardia). (**F**) Typical examples of left ventricular (LV) activation maps (stimulation at 120 ms) obtained by optical mapping. (**G**) Aged 129P2-MUT mice display significantly longer LV activation times, indicating more pronounced conduction slowing. (**H**) Typical example of a VT induced by 1 short-coupled extra stimulus (S1) in an isolated aged 129P2-MUT heart, and non-inducibility in an aged FVB/N-MUT heart with up to 3 extra stimuli (S1-S3). (**I**) Isolated aged 129P2-MUT hearts display significantly higher inducibility of ventricular arrhythmias (Pearson Chi-square overall *p*=0.03). Panel D: median(IQR); all other: mean±SD.

Figure 3. Cardiac hypertrophy induced by transaortic constriction (TAC) causes SCD and conduction disturbances in *Scn5a*$^{1798insD/+}$ mice. (**A**) Kaplan-Meier survival curves of WT and MUT mice subjected to Sham or TAC. (**B,C**) Magnitude of cardiac hypertrophy (measured by heart weight/tibia length ratio (B) and mRNA expression levels of the pro-hypertrophic marker *Rcan1-4* (C)) secondary to TAC is similar in WT and MUT mice. (**D**) Typical examples of atrio-ventricular (AV) delay measurements (atrial stimulation, 120 ms). (**E**) MUT-TAC mice display more severe AV-conduction delay versus WT-TAC. (**F**) Typical examples of LV activation maps (stimulation at 120 ms). (**G**) MUT-TAC mice display increased LV activation time. Panel G: median(IQR); all other: mean±SD. Additional data is presented in Supplementary Table 2.

Figure 4. Rescue of TAC-induced SCD and conduction disturbances in *Scn5a*$^{1798insD/+}$ mice by genetic deletion of *Nfatc2* or late sodium current inhibition by Ranolazine. (**A**) Kaplan-

Meier survival curves of WT and MUT mice (*Nfatc2*$^{-/-}$: with genetic deletion of *Nfatc2*; RAN: fed Ranolazine chow) subjected to Sham or TAC. (**B,C**) Magnitude of cardiac hypertrophy (measured by heart weight/tibia length ratio (B) and mRNA expression levels of *Rcan1-4* (C)) secondary to TAC is similar in WT and MUT (+/- Ranolazine chow) mice, but lower in WT and MUT mice on a *Nfatc2*$^{-/-}$ background. (**D**) Typical examples of atrio-ventricular (AV) delay measurements (atrial stimulation at 120 ms). The AV-conduction delay induced by TAC in MUT mice is reversed by genetic inhibition of *Nfatc2* and by Ranolazine. (**E**) Typical examples of LV activation maps in isolated hearts. (**F**) Increased LV activation time induced by TAC in MUT mice is reversed by genetic inhibition of *Nfatc2* and by Ranolazine. In panels **B**,**C**,**D** and **F**, data is presented as ratio for TAC versus Sham (mean±SD); actual values are in Supplementary Tables 2 and 4.

Table 1. Characteristics of *SCN5A*-1795insD mutation-positive patients suffering a serious cardiac event above the age of 40 with available recent clinical information

Gender	Age	PM	Event	Hypertension	Hypertension-related cardiac findings	Comments/other findings
Male	45	Yes	SCD	Yes	Postmortem: HW 490 gr, concentric LVH, slight interstitial myocardial fibrosis	SCD (VF documented) Postmortem: <50% stenosing coronary atherosclerosis; small foci of subendocardial necrosis; no valvar stenosis
Female	55	Yes	SCD	Borderline	MRI: LVH	SCD at night; previously documented VT
Female	46	Yes	SCD	Yes	Postmortem: HW 550 gr, concentric LVH, interstitial fibrosis	SCD at night Postmortem: minimal coronary atherosclerosis; no valvar stenosis
Female	49	Yes	SCD	Yes	Postmortem: HW 470 gr, concentric LVH, slight interstitial fibrosis	SCD at night Postmortem: minimal coronary atherosclerosis; no valvar stenosis
Female	41	Yes	VF	No*	Echo: marginal LVH	VF (resuscitated successfully)
Female	50	Yes	SCD	Yes	Echo: possible DCM	SCD (VF documented)
Female	58	Yes	VT	Yes	Echo: diastolic dysfunction	VT documented at night necessitating ICD implantation
Female	69	No	VT/VF	Yes	Unknown	Multiple episodes of syncope; documented polymorphic VTs
Male	60	No	SCD	Yes	Unknown	Normal echo 1 year prior to SCD
Male	69	No	SCD	Yes	Postmortem: HW 530 gr, concentric LVH	SCD (VF documented) Postmortem: ischemic scar; no signs of recent-onset ischemia; no valvar stenosis

PM: pacemaker; DCM: dilated cardiomyopathy; ICD: implantable cardioverter defibrillator; LVH: left ventricular hypertrophy; SCD: sudden cardiac death; VF: ventricular fibrillation; VT: ventricular tachycardia

*Patient did not have documented hypertension prior to VF, but developed clinically relevant hypertension one year later

Figure 1

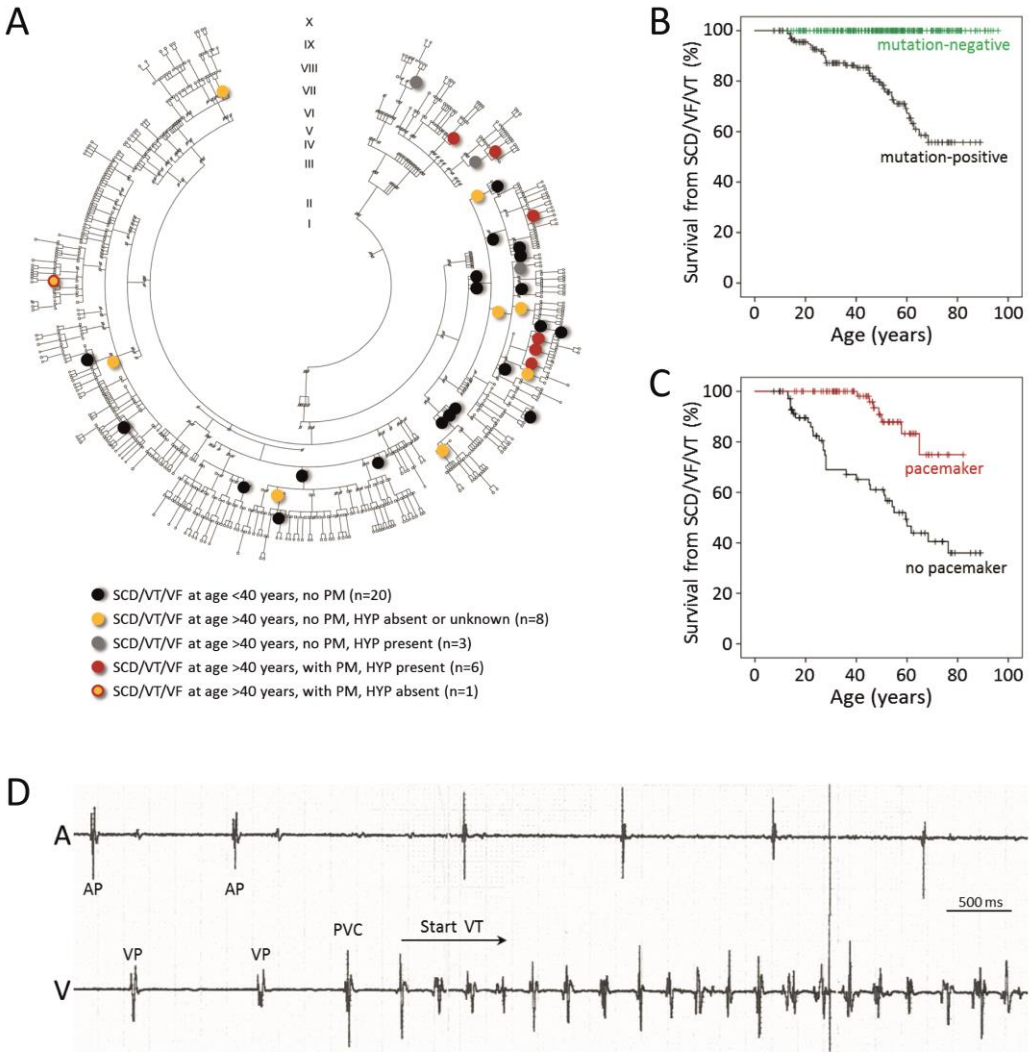


Figure 1

Figure 2

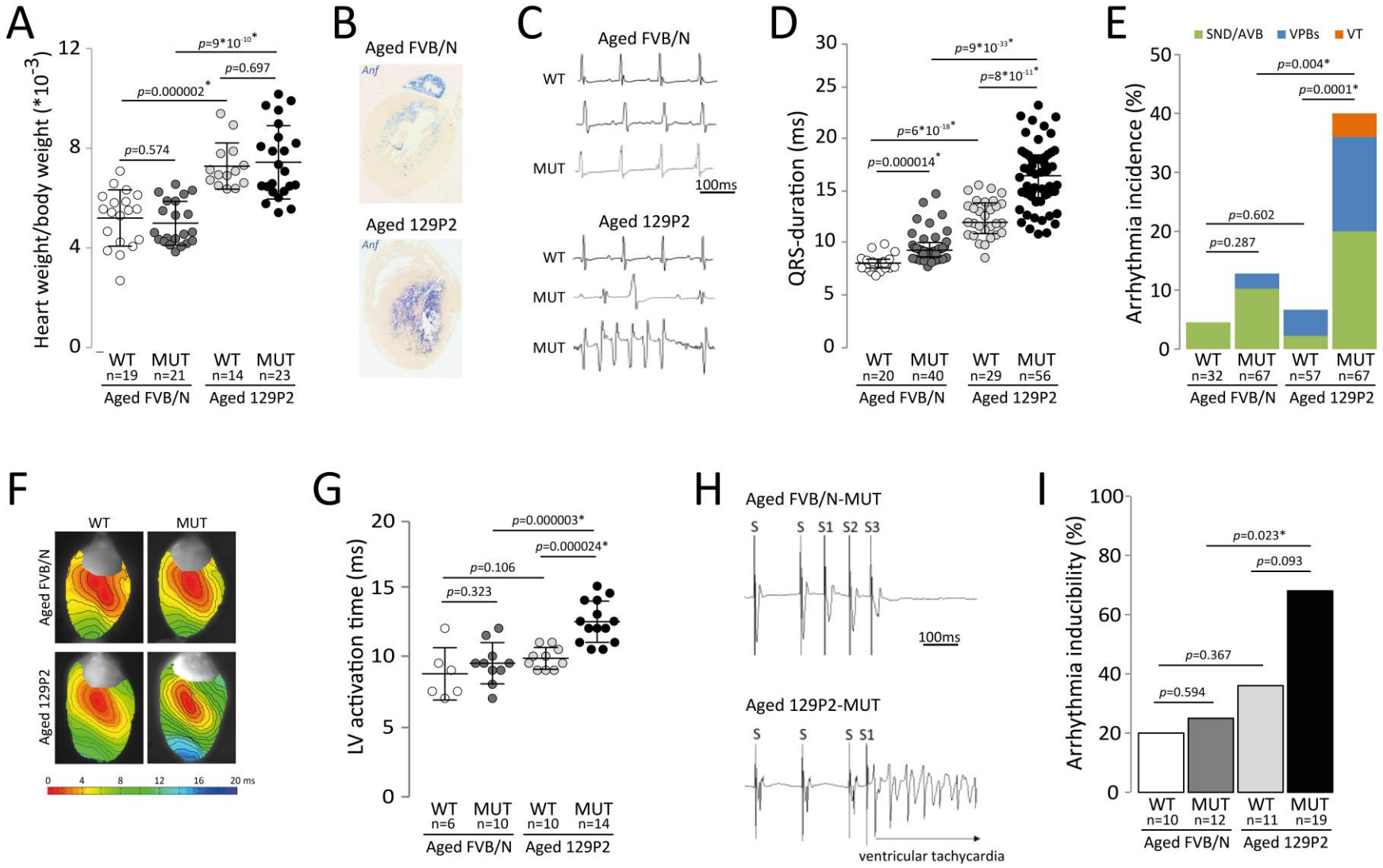

A
Heart weight/body weight (*10-3)
p=0.574
p=0.000002*
p=0.697
p=9*10-10*
WT n=19
MUT n=21
WT n=14
MUT n=23
Aged FVB/N
Aged 129P2
B
Aged FVB/N
Anf
Aged 129P2
Anf
C
Aged FVB/N
WT
MUT
100ms
Aged 129P2
WT
MUT
MUT
D
QRS-duration (ms)
p=0.000014*
p=6*10-18*
p=8*10-11*
p=9*10-33*
WT n=20
MUT n=40
WT n=29
MUT n=56
Aged FVB/N
Aged 129P2
E
SND/AVB
VPBs
VT
Arrhythmia incidence (%)
p=0.287
p=0.602
p=0.004*
p=0.0001*
WT n=32
MUT n=67
WT n=57
MUT n=67
Aged FVB/N
Aged 129P2
F
WT
MUT
Aged FVB/N
Aged 129P2
0 4 8 12 16 20 ms
G
LV activation time (ms)
p=0.323
p=0.106
p=0.000024*
p=0.000003*
WT n=6
MUT n=10
WT n=10
MUT n=14
Aged FVB/N
Aged 129P2
H
Aged FVB/N-MUT
S S S1 S2 S3
100ms
Aged 129P2-MUT
S S S S1
ventricular tachycardia
I
Arrhythmia inducibility (%)
p=0.594
p=0.367
p=0.093
p=0.023*
WT n=10
MUT n=12
WT n=11
MUT n=19
Aged FVB/N
Aged 129P2

Figure 3

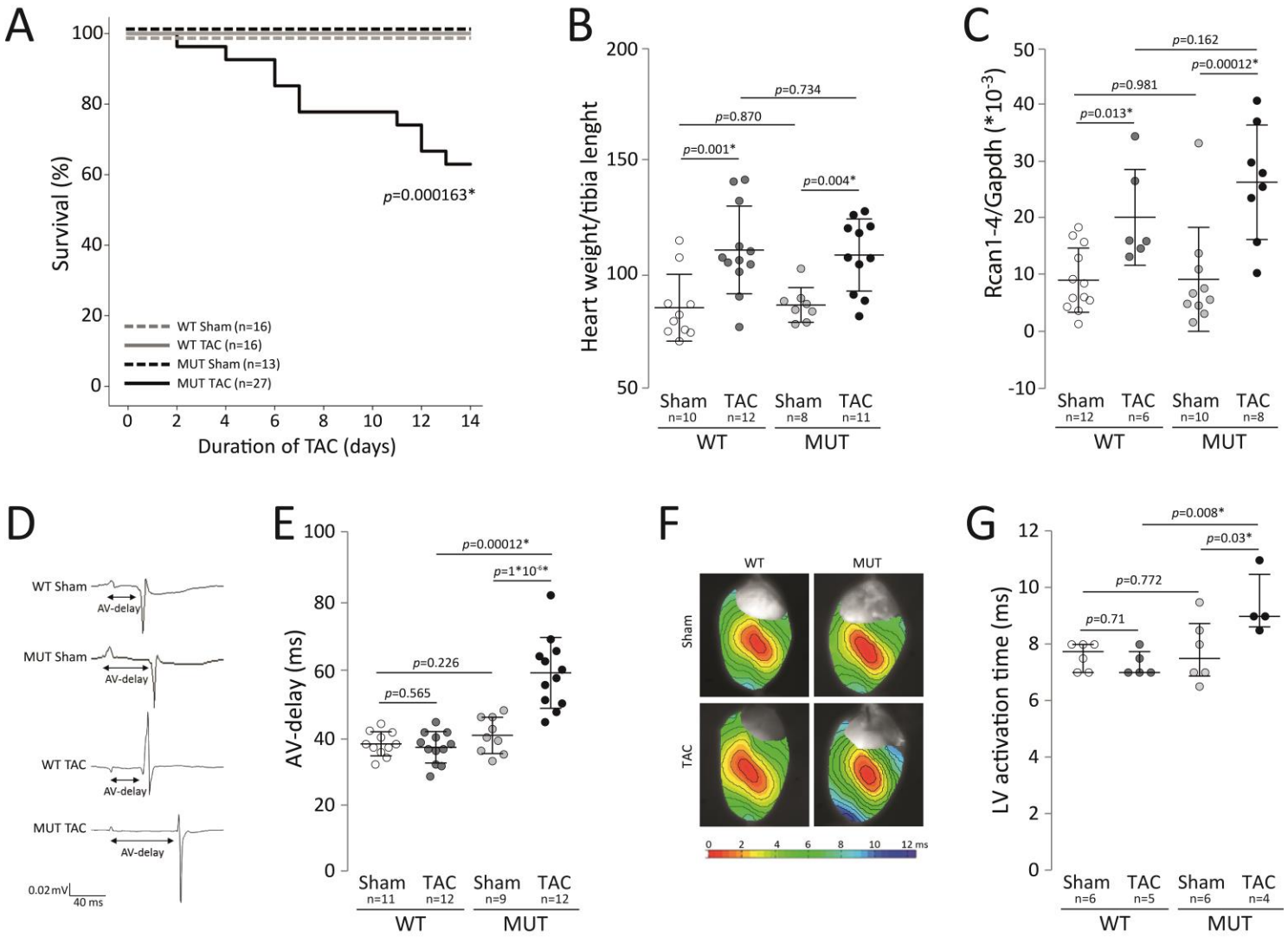
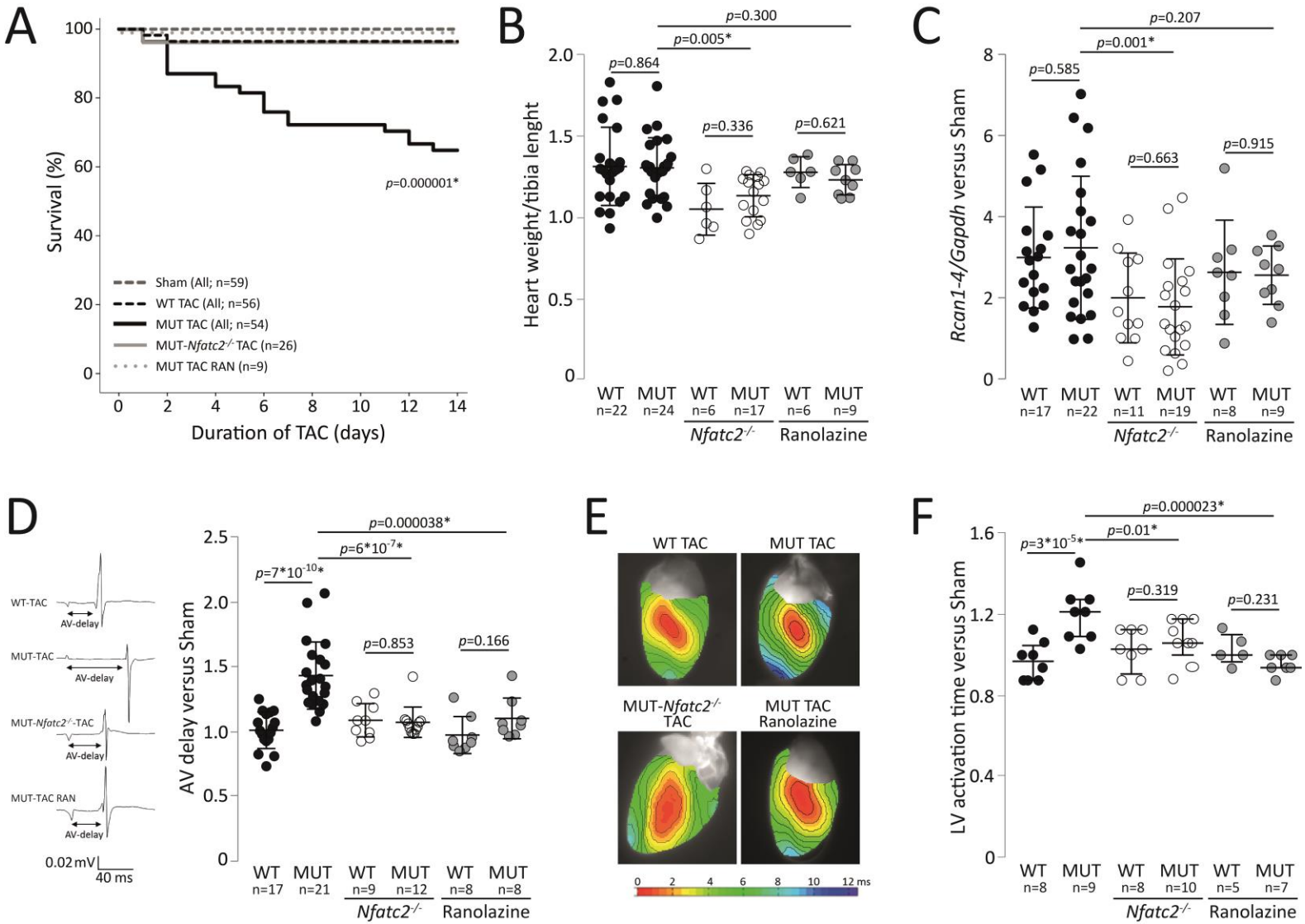

A
Survival (%)
Duration of TAC (days)
p=0.000163*
WT Sham (n=16)
WT TAC (n=16)
MUT Sham (n=13)
MUT TAC (n=27)
B
Heart weight/tibia lenght
p=0.001*
p=0.870
p=0.734
p=0.004*
Sham n=10
TAC n=12
Sham n=8
TAC n=11
WT
MUT
C
Rcan1-4/Gapdh (*10-3)
p=0.013*
p=0.981
p=0.162
p=0.00012*
Sham n=12
TAC n=6
Sham n=10
TAC n=8
WT
MUT
D
WT Sham
MUT Sham
WT TAC
MUT TAC
AV-delay
0.02 mV
40 ms
E
AV-delay (ms)
p=0.565
p=0.226
p=0.00012*
p=1*10-6**
Sham n=11
TAC n=12
Sham n=9
TAC n=12
WT
MUT
F
WT
MUT
Sham
TAC
0 2 4 6 8 10 12 ms
G
LV activation time (ms)
p=0.71
p=0.772
p=0.008*
p=0.03*
Sham n=6
TAC n=5
Sham n=6
TAC n=4
WT
MUT

Figure 4


A
Survival (%)
Duration of TAC (days)
p=0.000001*
Sham (All; n=59)
WT TAC (All; n=56)
MUT TAC (All; n=54)
MUT-Nfatc2-/- TAC (n=26)
MUT TAC RAN (n=9)
B
Heart weight/tibia lenght
p=0.300
p=0.005*
p=0.864
p=0.336
p=0.621
WT n=22 MUT n=24 WT n=6 MUT n=17 WT n=6 MUT n=9
Nfatc2-/- Ranolazine
C
Rcan1-4/Gapdh versus Sham
p=0.207
p=0.001*
p=0.585
p=0.663
p=0.915
WT n=17 MUT n=22 WT n=11 MUT n=19 WT n=8 MUT n=9
Nfatc2-/- Ranolazine
D
WT-TAC
MUT-TAC
MUT-Nfatc2-/-TAC
MUT-TAC RAN
AV-delay
0.02 mV
40 ms
AV delay versus Sham
p=0.000038*
p=6*10-7*
p=7*10-10*
p=0.853
p=0.166
WT n=17 MUT n=21 WT n=9 MUT n=12 WT n=8 MUT n=8
Nfatc2-/- Ranolazine
E
WT TAC
MUT TAC
MUT-Nfatc2-/- TAC
MUT TAC Ranolazine
0 2 4 6 8 10 12 ms
F
LV activation time versus Sham
p=0.000023*
p=0.01*
p=3*10-5*
p=0.319
p=0.231
WT n=8 MUT n=9 WT n=8 MUT n=10 WT n=5 MUT n=7
Nfatc2-/- Ranolazine